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FOREWORD

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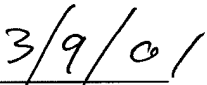
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INTRODUCTION

The objective of this case-control study is to determine whether oxidative damage is a risk factor for prostate cancer, and whether this mechanism mediates the association between dietary fat and prostate cancer risk. Specifically, cases and controls will be compared with respect to malondialdehyde (MDA) in serum as a measure of oxidative stress, and deoxyguanosine malondialdehyde (dG-MDA) in peripheral lymphocytes and prostate tumor samples as a measure of oxidative DNA damage. In addition to these measures, dietary intake of fats and specific fatty acids, and of antioxidants will be considered as potential effect modifiers or confounding factors, as will serum antioxidant levels.

This report covers primarily startup and patient accrual activities during the first year of the project. These activities include finalizing the clinical protocol for identifying, enrolling, and obtaining samples from cases and controls, and implementing regular enrollment of subjects, and preliminary analysis of samples. In addition, the report will describe changes to take place during the 2nd year of the award, related to the Principal Investigator's move from Georgetown University Medical Center to the Johns Hopkins Medical Institutions, Brady Urological Institute.

BODY

1. Study Progress

Progress in the study during the first year of funding will be described below with respect to each of the tasks in the original Statement of Work (only those tasks expected to begin during Year 1 will be described).

Task 1. To enroll prostate cancer cases scheduled to undergo prostatectomy, and benign urologic surgery controls, from urology departments of four major hospitals in the Washington, DC metropolitan area (months 1-30).

Initial efforts involved optimizing the process of enrolling the patients and obtaining data and samples from the clinics. Because the Project Manager needed to be directly integrated into busy clinical practices, we initially focused on optimal enrollment of cancer cases. From discussions with the collaborating urologists it became clear that the nature of the clinic visit would be most complicated for the patients with cancer. We decided that the Project Manager would initially focus on enrolling patients at the time of biopsy, or those returning to the clinic following their diagnosis to decide on a treatment plan. This would maximize our enrollment of true cases, allow us to identify patients with PIN whose biology may be intermediate between cases and controls, and identify some controls (i.e. those with normal or BPH on biopsy with PSA < 2.5).

We are currently enrolling patients at three of the planned four hospitals: Georgetown University Medical Center (GUMC), the Veterans Administration Hospital (VAH), and the Washington Hospital Center (WHC). Enrollment at the fourth hospital (George Washington University Hospital – GWU) has not begun due to ongoing institutional changes. However, enrollment from the three hospitals has been very good (data through 1/19/01):

| | |
|---|------------|
| Prostate cancer cases | 94 |
| Patients with BPH | 50 |
| Patients with PIN | 51 |
| Patients with normal or non-neoplastic pathology | 22 |
| Patients with pathology results pending | 71 |
| Controls (no biopsy) | 5 |
| Total patients enrolled: | 293 |

Some of the BPH patients and those with normal/non neoplastic pathology will be eligible to be controls. We are currently abstracting their PSA data from the medical records. Now that enrollment of prostate cancer cases is running smoothly, we have begun to also enroll men attending the clinic for conditions unrelated to cancer (such as kidney stones). We are increasing

our efforts in this area so that control enrollment can keep pace of case enrollment. The reason that the number of patients is so high with pathology listed as "pending" is that, for the WHC patients, we have been waiting until there were a large number of subjects before pulling medical records to abstract the pathology report. All of the patients from this site come from the practice of a single physician. To minimize disruption of his clinic operation, we have worked out an arrangement that the Project Manager will come to his clinic on a day when he is not there to abstract a large number of records.

The ethnic distribution of the patients is 57.7% white/non-Hispanic, 39.3% African-American, 3.0% other.

Task 2. To measure the biomarkers related to oxidative stress, fatty acids and antioxidants and hormonal profiles (months 1-30).

Task 3. To conduct a case-control study with the above data to determine the individual and joint roles of oxidative stress and dietary fat on prostate cancer risk (months 1-36).

Progress on Tasks 2 and 3 is considered together as the biomarker measurements are compared between cases and controls.

As a preliminary analysis, we sent serum samples for 14 case-control pairs to our collaborator at the University of Toronto, Dr. A. Venket Rao. All controls had BPH. The focus was on measures of malondialdehyde (MDA), and the antioxidant profile. Cases and controls were matched on age (± 5 years) and race. These samples were largely from the VAH patient population, so the ethnic distribution was predominantly African American (10 pairs African American; 4 pairs Caucasian). Paired t-tests were conducted for variables that were normally distributed. In the absence of normality, the data was log-transformed to establish normality and paired t-tests were done on the transformed data. If log-transformation was unsuccessful, the non-parametric Wilcoxon Signed-Rank test was used.

Statistically significant differences between cases and controls were observed for serum MDA, lutein, and α -carotene (see table below). Variables that did not exhibit statistically significant differences were lycopene, cryptoxanthin, retinol, β -carotene, and α -tocopherol (data not shown).

| | Range | Cases Mean (SD) | Controls Mean (SD) | p-value |
|-------------------------|---------------|--------------------|-----------------------|---------|
| MDA (μ M) | 2.4 – 3.9 | 2.9 (0.26) | 3.2 (0.37) | 0.03 |
| lutein (nM) | 107.8 – 899.3 | 428.7 (192.73) | 673.5 (123.4) | 0.0004 |
| α -carotene (nM) | 0.0 – 90.5 | 11.2 (14.90) | 31.63 (32.90) | 0.035 |

As can be seen, cases had lower values than controls for each of the three measures. Although the sample size is too small to attach any clear interpretation to the results, the associations with lutein and α -carotene are consistent with a hypothesized protective effect of antioxidants. However, for MDA, the effect is contrary to that expected, i.e. lower levels of lipid peroxidation in cases. A finding of lower levels of lipid peroxidation in cancer cases has been seen in another study of multiple cancer sites, including prostate (Gerber 1996). This was attributed to the depletion of membrane polynunsaturated fatty acids in more advanced cancer and in highly anaplastic cells. This underscores the need to focus on early stage tumors. Because we have also enrolled men with PIN, we will compare them to cases and controls to determine whether associations differ at a point early in the neoplastic process. Furthermore, when we analyse a larger number of samples, we will adjust for tumor stage, size, and Gleason grade to determine whether these factors modify the association between lipid peroxidation and prostate cancer risk.

Dr. Rao noted that the levels of serum lycopene (control mean=259.0 nM, case mean=240.8 nM) are much lower than what he usually observes in healthy subjects (means > 500 nM). Furthermore, serum MDA levels are higher than he usually observes in healthy subjects (1.2–2.0 μ M). The possible reasons for discrepancy include (a) the small sample size, (b) patients at the VAH often have significant co-morbidity, particularly alcohol abuse, and may have poor nutrient balance, or (c) some of the controls (who all have biopsy-proven BPH) may have undiagnosed prostate cancer. All of these possibilities will be evaluated in subsequent analyses based on a larger, more diverse patient sample.

In addition to the above results, MDA was significantly correlated with retinol, but not with any of the other nutrients. None of the variables were significantly correlated with age (age range: 46-80). There was a marginally non-significant correlation between lycopene levels (log transformed) and PSA, $r = 0.34$, $p = 0.10$. This association has previously been observed in prostate cancer studies by Dr. Rao.

We have not performed analyses of androgen profiles in the 28 samples. In the original grant we proposed that these analyses would also be performed by Dr. Rao. However, prior to the awarding of the grant, we decided to have these analyses conducted here at GUMC by the Bioanalytical Core Facility. This Core has extensive experience with the proposed assays (this change and the re-apportioning of the funds requested for assays were described in a letter that was sent by Dr. Trock to Sherry Regalado, Contract Specialist for the US Army Medical Research Acquisition Activity, dated November 10, 1999). However, because of the large volume of assays done by the Bioanalytical Core Facility for investigators throughout GUMC, they will wait until we have larger batches of samples to be analysed simultaneously to allow more efficient processing. We are waiting until we have 80 case-control pairs (one-third of the total study size) before processing samples for the androgen profile. This should be attained

during Year 2.

To date we have not retrieved tissue samples from the participating pathology departments. Although arrangements have been set up by discussions with pathologists at each of the institutions, we have decided to wait until a large number of samples can all be pulled at the same time. Since there is no money in the grant to pay the pathologists for pulling samples and reviewing slides to ensure that samples contain tumor, we are trying to make things more efficient by setting aside time when a large batch can be retrieved. We are now at that point and lists of samples to be pulled are being prepared now and will be submitted to the pathologists shortly.

2. Change in Institution in Year 2

In April 2001, the PI of the study, Dr. Bruce Trock, will leave GUMC to take a position at the Johns Hopkins Medical Institutions, Brady Urological Institute. The current award will be transferred to Johns Hopkins and the study will continue. The staff supported on the grant, Michelle Brotzman and Patricia Kolmer (who replaced Maria Malone), will also relocate with Dr. Trock and continue to work on the study. The Brady Urological Institute (BUI) is one of the largest urological practices in the country, particularly in the area of prostate cancer, performing over 800 prostatectomies annually. Dr. Trock will be working directly with the urologists, and establishing epidemiologic studies at the BUI is one of his primary charges, so there will be no trouble continuing to accrue ample numbers of patients to the study. Prostate cancer patients come to the BUI from all over the world, so there is the potential for a selection bias in the patient population. To minimize the likelihood of such bias, patients from the BUI will only be enrolled if they live in the Baltimore metropolitan area, i.e. those patients who are clearly within the normal catchment area of a large hospital. There is also a large screening population in the east Baltimore area where the BUI is located, which is part of the Baltimore Longitudinal Study of Aging. This population will be a good source of healthy, predominantly African American, controls. Furthermore, patient enrollment at the VAH will continue, to insure that there is ample representation of African American men in the study. The Project Manager will continue with the current protocol of going to the VAH every Friday, the day that biopsies are performed, to enroll patients. Patients with other urological conditions are also seen at this clinic so control enrollment will also continue unchanged. Thus, the study is likely to actually be enhanced by the move.

CONCLUSION

Patient and sample accrual has proceeded very well during Year 1 of the award, and despite implementing enrollment at three of the planned four institutions, there should be no trouble

achieving the projected sample size for the study. Preliminary analyses of MDA and antioxidant profiles (primarily among patients from VAH) show that MDA levels are higher, and antioxidant levels lower, than have often been reported for healthy individuals. Based on the small sample of 14 matched case-control pairs, MDA was lower in cases than controls, as were the antioxidants lutein and α -carotene. While the latter is consistent with a hypothesized protective effect of antioxidants, the former is contrary to the primary study hypothesis of lipid peroxidation as a marker of increased risk. However, these results could reflect random variability due to a small sample, poor nutrient balance associated with co-morbidity in VAH patients, undiagnosed prostate cancer in the controls, who all had biopsy-proven BPH, or depletion of substrate membrane polyunsaturated fatty acids associated with more advanced/less differentiated tumors. All of these possibilities will be investigated when a larger number of samples are analysed. Analysis of serum samples for androgen profiles, and of prostate tissue samples for dG-MDA will begin in Year 2, to allow for greater efficiency by processing larger batches of samples. Finally, in April 2001, the study will move with Dr. Trock to the Johns Hopkins Medical Institutions, where it will continue amidst a larger potential patient population, although enrollment at the VAH will continue.

REFERENCES

Gerber M, Astre C, et al. Oxidant-antioxidant status alterations in cancer patients: relationship to tumor progression. J Nutr 1996; 126 (suppl 4):1201s-1207s.

APPENDICES

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LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|--------|---------------------------------------|
| GUMC | Georgetown University Medical Center |
| VAH | Veterans Administration Hospital |
| WHC | Washington Hospital Center |
| GWU | George Washington University Hospital |
| BPH | Benign prostatic hypertrophy |
| PIN | Prostatic intraepithelial neoplasia |
| PSA | Prostate-specific antigen |
| MDA | malondialdehyde |
| dG-MDA | deoxyguanosine malondialdehyde |
| BUI | Brady Urological Institute |

Meeting abstracts during reporting period: None in connection with this project

Publications during reporting period: None in connection with this project

Manuscripts in preparation: None in connection with this project

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